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Research Article

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cDNA Sequence and mRNA Expression of a Novel Serine Protease from the Firefly, *Pyrocoelia rufa*

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We describe here the cDNA sequence and mRNA expression of a novel serine protease from the firefly, *Pyrocoelia rufa*. The 771 bp cDNA encodes for 257 amino acid residues. The deduced protein of *P. rufa* serine protease gene contains the catalytic triad and six-conserved cysteine residues. Alignment of the deduced protein of *P. rufa* serine protease gene showed 47.4% protein sequence identity to known coleopteran insect *Rhyzopertha dominica* midgut trpsin-like enzyme. Northern blot analysis revealed that the *P. rufa* serine protease is specifically expressed in the midgut of *P. rufa* larvae.

Key words: Insect, Firefly, Serine protease, *Pyrocoelia rufa*, cDNA sequence, Phylogeny

Introduction

Serine proteases are major insect gut enzymes involved in digestion of dietary proteins, and they play critical roles in a variety of invertebrate immune processes. Serine proteases in defense responses have been implicated in hemolymph coagulation (Iwanaga *et al.*, 1998), activation of antimicrobial peptide synthesis (Imler and Hoffmann, 2000; Jiang and Knost, 2000), and melanin synthesis (Ashida and Brey, 1997; Jiang and Kanost, 2000).

Serine proteases are involved in the blood meal digestion in haematophogous insects. Much of the recent study has been carried out in several vector insects, which are medically important in animals and humans (Gorman *et al.*, 2000; Lehane *et al.*, 1998; Paskewitz and Gorman, 1999; Yan *et al.*, 2001). Despite the important of these serine proteases in invertebrate immunity, few of their genes have been cloned in coleopteran insects.

In this study, we report the cDNA sequence and mRNA expression of a novel family of the serine protease from the firefly, *Pyrocoelia rufa*. The characterization for novel family of serine protease in Coleoptera is discussed.

Materials and Methods

Insects

The larvae of the firefly, *Pyrocoelia rufa*, were collected at Miryang, Kyungnam Province in Korea. The live larvae were directly used in this study.

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library constructed from the whole body of *P. rufa* larvae (Lee *et al.*, 2000, 2001) was used in this study. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

MacVector (ver. 6.5) was used to align the amino acid sequences of serine protease gene. Including the twenty GenBank-registered amino acid sequences of serine protease genes, phylogenetic analysis among serine protease genes was performed using PAUP (Phylogenetic Analysis using Parsimony) version 3.0 (Swofford, 1990). The tree was obtained by bootstrap analysis with the option of heuristic search (1,000 replications). Outgroup was chosen as

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Glossina morsitans morsitans serine protease gene on the basis of the sequence homology obtained by pairwise comparison. The accession numbers of the sequences in the GenBank are as follows: firefly *Pyrocoelia rufa* (this study), lesser grain borer *Rhyzopertha dominica* (AF 130841), *Anopheles stephensi* (U52359), *Anopheles gambiae* (Z22930), *Culex pipiens quinquefasciatus* (U65412, AY029276), *Aedes aegypti* (TRWV3Y), *Lucilia cuprina* (L15632), *Drosophila erecta* (U40653), *Drosophila melanogaster* (U04853), *Drosophila virilis* (U93213), early cattle grub *Hypoderma lineatum* (X74304), *Stomoxys calcitrans* (AF074956), grey fleshfly *Sarcophaga bullata* (P51588), Indian meal moth *Plodia interpunctella* (AF 074956), cotton bollworm *Helicoverpa armigera* (Y12283), spruce budworm *Choristoneura fumiferana* (U12917), tobacco hornworm *Manduca sexta* (T10109), cat flea *Ctenocephalides felis* (AF053918, AF053910), and *Glossina morsitans morsitans* (AF252868). The hydropathy plot of *P. rufa* serine protease was analyzed by the Kyte and Doolittle method (Kyte and Doolittle, 1982). Helical and non-helical regions of *P. rufa* serine protease were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

RNA isolation and Northern blot analysis

Ten larvae of *P. rufa* were dissected under the Stereomicroscope (Zeiss, Jena, Germany). Individual samples such as midgut, fat body, head and light organ were harvested and washed twice with PBS (140 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4). Total RNAs were isolated from the whole body, midgut, and fat body of the *P. rufa* larvae by using the Total RNA Extraction Kit (Promega, Madison, WI). Total RNAs (10 μg/lane) from the *P. rufa* were denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the serine protease gene transcripts was 771 bp serine protease gene cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C, and finally exposed to X-ray film. For rehybridization, the membrane was washed for 20 min at room temperature in sterile millipore water. Then the membrane was washed overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS in order to remove the hybridized probe. The membrane was then rehybridized to [α-^{32}P] dCTP-labeled 16S rRNA probe (Lee *et al.*, 2000). The 16S rRNA gene was used as an internal loading control.

Results and Discussion

A cDNA library prepared from the whole body of *P. rufa* larvae (Lee *et al.*, 2000) was screened. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *P. rufa* ESTs. Of these ESTs, one exhibited similarity to the reported serine protease genes. The nucleotide and deduced amino acid sequences of a full-length cDNA encoding the *P. rufa* serine protease is presented in Fig. 1. The complete serine protease cDNA sequence comprised of 771 bp with 257 amino acid residues.

A multiple sequence alignment of the deduced protein sequence of *P. rufa* serine protease gene with five other serine protease sequences is shown in Fig. 2. Alignment of the *P. rufa* serine protease sequences with those for serine protease from several other species indicates the extent of the identity that exists. The deduced protein sequences represent prepro peptides with hydrophobic signal peptide sequences associated with their 5-terminus (Fig. 3). The five á helices of *P. rufa* serine protease are

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  1 ATGTTCAAAATTGTGCTTATTGCAGCTCTCGCGGCTCTTGCCGTCGACGCAAGGTTCCTT
  1 M  F  K  I  V  L  I  A  A  L  A  A  L  A  V  D  A  R  F  L

 61 TCACGAGCTCCCCAACTAGATGGAAGAATTGTCGGTGGCAAAGATACAACCATTGAAGAT
 21 S  R  A  P  G  L  D  G  R  I  V  G  G  K  D  T  T  I  E  D

121 TTTCCGCATCAAGTTTCTCTTCAACTATACGGTGGACACGCTTGCGGTGGATCGATTATC
 41 F  P  H  Q  V  S  L  Q  L  Y  G  G  H  A  C  G  G  S  I  I

181 GCTAGCAACATAATTTTGACAGCTGCTCACTGCACGCACTTACGTTCAGCCCGTATCATG
 61 A  S  N  I  I  F  T  A  A  H  C  T  H  L  R  S  A  R  I  M

241 AGCATTCGTTACGGTAGCAGTATAATGGATGACGAAGGTACAGTTATGGACGTATCTGAG
 81 S  I  R  Y  G  S  S  I  M  D  D  E  G  T  V  M  D  V  S  E

301 GTACTTCAACATCCAAGCTACAACCCAGCGACCACCGATTATGACATTTCACTGTTAATA
101 V  L  Q  H  P  S  Y  N  P  A  T  T  D  Y  D  I  S  L  L  I

361 TTAGATGGAAGCGTGGTGTTGTCACACAAGGCTCAGATCATTAATTTGGTTCCCTCAAAA
121 L  D  G  S  V  V  L  S  H  K  A  Q  I  I  N  L  V  P  S  K

421 TCACCAGAAGGAGGTCGTAGCGCTTTTGTAACCGGATGGGGAGCCATTTACTCAGGTGGC
141 S  P  E  G  G  R  S  A  F  V  T  G  W  G  A  I  Y  S  G  G

481 CCAGCATCGAAACAGTTACAAGTTGTTGAAGTCAACGAGGAAGATAGAGAGGCATGTAAA
161 P  A  S  K  Q  L  Q  V  V  E  V  N  E  E  D  R  E  A  C  K

541 AGCGCATATGATGGAGACATTACCGAAAGAATGATCTGCTTCAAGGATGCCGGTCAAGAC
181 S  A  Y  D  G  D  I  T  E  R  M  I  C  F  K  D  A  G  Q  D

601 TCATGTCAGGGAGATTCTGGTGGCCCACTCGTATCTAGCGATGGACAGATTGGGGTAGTT
201 S  C  Q  G  D  S  G  G  P  L  V  S  S  D  G  Q  I  G  V  V

661 TCATGGGGGTACGGATGCGCAGATCCCAGATATCCTGGAGTATATTCACATGTTGATAAC
221 S  W  G  Y  G  C  A  D  P  R  Y  P  G  V  Y  S  H  V  D  N

721 GAGCACCTCAGGGAGTACATTGAATCCAATGTAAAAGTGCATAGTAGAAAATAAGAATGA
241 E  H  L  R  E  Y  I  E  S  N  V  K  V  H  S  R  K  *

781 CATTGTGTTAACAAAGTTAATATTCAATTTATATTATTCAATTTATTCTTTTGTAACCAT
841 AGCAATAAATACTGGAAAAAAACGTCAAAAAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *P. rufa* serine protease gene. The start codon of ATG is boxed and the termination codon is asterisked. Six cysteine residues are marked by solid circles. A polyadenylation signal is underlined.

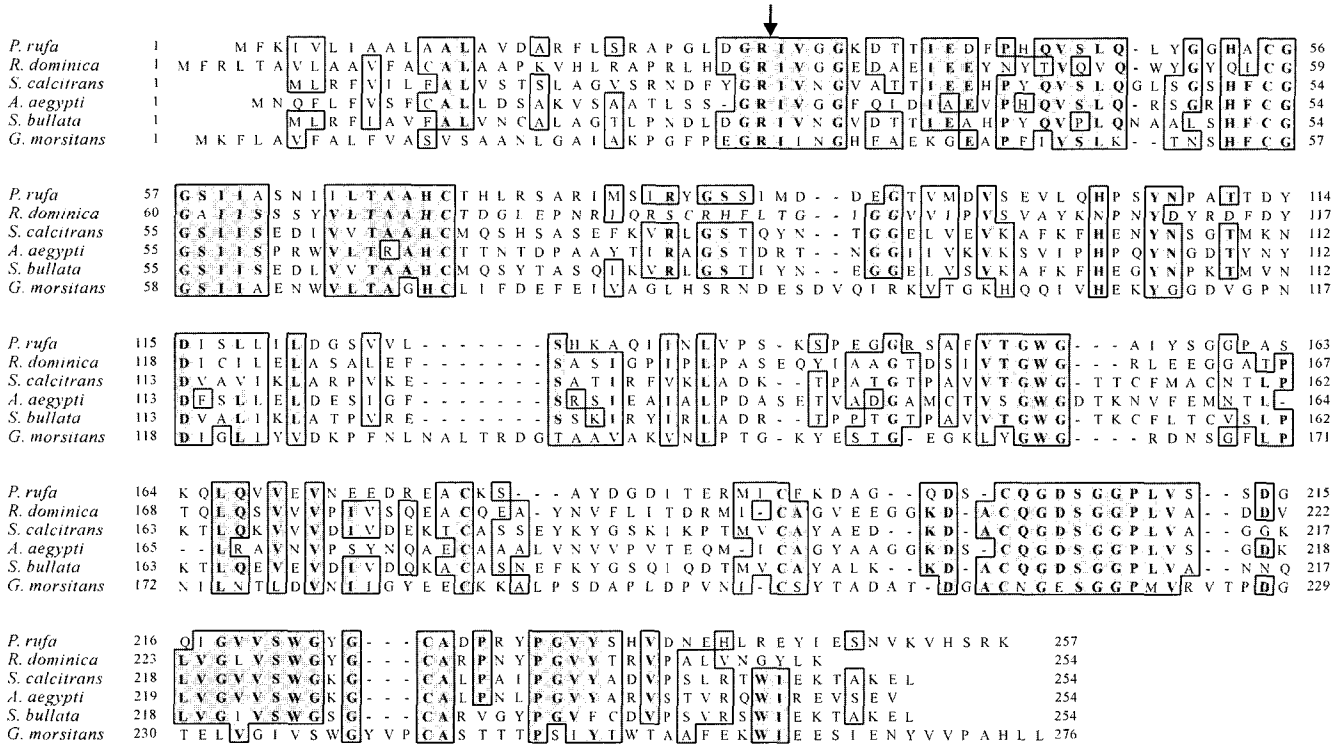

Fig. 2. Alignment of deduced amino acid sequences of *P. rufa* serine protease gene with other known serine proteases. In solid box are the residues that are identical to those in *P. rufa* serine protease. The beginning site of the mature peptide is indicated by arrow. The residues of the catalytic triad (H, D, S) in the *P. rufa* serine protease are indicated by crosses. Six conserved cysteine residues are marked by asterisks.

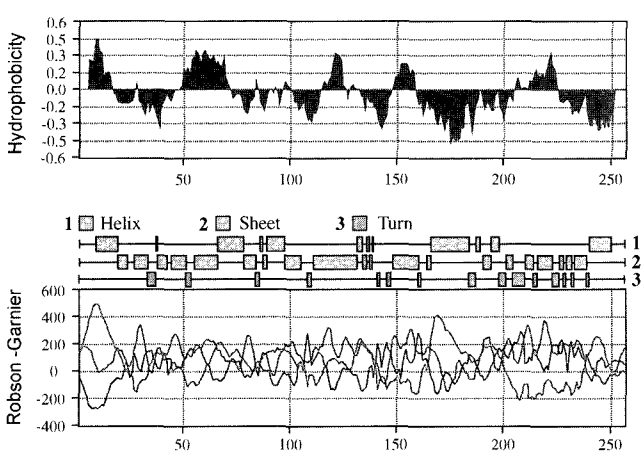

Fig. 3. The hydropathy profile (upper panel) and predicted helical regions (lower panel) of *P. rufa* serine protease. Hydropathic analysis was done as described by Kyte and Doolittle (1982). Helical and non-helical regions were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

predicted as shown in Fig. 3. The cleavage site of the mature proteins has been identified based on the conserved cleavage site of the putative activation peptide between an arginine and isoleucin residue (arrow in Fig. 2). It is present at the 5-end of various mature serine proteases (Han *et al.*, 1997; Yan *et al.*, 2001). Therefore, the result suggests that the predicted size of the 228 amino acids mature peptide in *P. rufa* serine protease is about 25,000 Da. The alignment of these six sequences shows that they have the structural requirements of serine proteases such as the catalytic triad and six conserved cysteine residues. All of these six sequences harbour the catalytic triad, His, Asp and Ser (Kraut, 1977; Lehane *et al.*, 1998; Yan *et al.*, 2001), and the six highly conserved Cys residues to allow the formation of the three disulfide bonds which are typical of invertebrate serine proteases (Lehane *et al.*, 1998; Yan *et al.*, 2001).

The *P. rufa* serine protease showed 47.4% protein sequence identity to the *R. dominica* midgut trpsin-like enzyme (Table 1). Furthermore, *P. rufa* serine protease shows sequence similarity to other known serine proteases from a wide variety of species.

A phylogenetic tree was constructed using the protein sequences of known serine protease genes (Fig. 4). The phylogenetic analysis revealed that the deduced protein sequence of *P. rufa* serine protease gene separated with a coleopteran insect *R. dominica* serine protease from other

Table 1. Pairwise comparison among amino acid sequences of the *P. rufa* serine protease gene and the known serine protease genes

Species	GenBank no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. *P. rufa*		–	0.526	0.59	0.593	0.554	0.548	0.516	0.487	0.484	0.487	0.484	0.49	0.529	0.522	0.609	0.603	0.593	0.59	0.5	0.51	0.679
2. *R. dominica*	AF130841	164	–	0.564	0.564	0.519	0.529	0.529	0.554	0.519	0.51	0.535	0.548	0.551	0.542	0.593	0.593	0.574	0.574	0.545	0.548	0.715
3. *A. stephensi*	U52359	184	176	–	0.208	0.449	0.404	0.481	0.551	0.554	0.558	0.548	0.564	0.596	0.599	0.631	0.631	0.628	0.599	0.538	0.526	0.747
4. *A. gambiae*	Z22930	185	176	65	–	0.442	0.433	0.478	0.545	0.554	0.564	0.558	0.571	0.593	0.603	0.609	0.622	0.612	0.596	0.551	0.554	0.756
5. *C. pipiens*	U65412	173	162	140	138	–	0.378	0.452	0.548	0.529	0.529	0.522	0.522	0.548	0.554	0.596	0.615	0.619	0.615	0.497	0.468	0.696
6. *C. pipiens*	AY029276	171	165	126	135	118	–	0.413	0.538	0.529	0.542	0.542	0.522	0.571	0.583	0.609	0.596	0.609	0.593	0.5	0.506	0.708
7. *A. aegypti*	TRWV3Y	161	165	150	149	141	129	–	0.51	0.494	0.503	0.503	0.513	0.526	0.558	0.564	0.567	0.571	0.554	0.5	0.494	0.67
8. *L. cuprina*	L15632	152	173	172	170	171	168	159	–	0.215	0.215	0.192	0.356	0.388	0.42	0.554	0.526	0.554	0.564	0.548	0.554	0.679
9. *D. erecta*	U40653	151	162	173	173	165	165	154	67	–	0.093	0.138	0.356	0.394	0.41	0.545	0.535	0.548	0.558	0.548	0.548	0.679
10. *D. melanogaster*	U04853	152	159	174	176	165	169	157	67	29	–	0.154	0.359	0.401	0.41	0.545	0.538	0.554	0.567	0.548	0.554	0.679
11. *D. virilis*	U93213	151	167	171	174	163	169	157	60	43	48	–	0.356	0.381	0.407	0.561	0.548	0.558	0.561	0.551	0.542	0.683
12. *H. lineatum*	X74304	153	171	176	178	163	163	160	111	111	112	111	–	0.426	0.429	0.574	0.59	0.599	0.59	0.542	0.545	0.686
13. *S. calcitrans*	AF074956	165	172	186	185	171	178	164	121	123	125	119	133	–	0.221	0.583	0.587	0.583	0.596	0.532	0.532	0.667
14. *S. bullata*	P51588	163	169	187	188	173	182	174	131	128	128	127	134	69	–	0.58	0.583	0.574	0.593	0.522	0.519	0.683
15. *P. interpunctella*	AF064526	190	185	197	190	186	190	176	173	170	170	175	179	182	181	–	0.324	0.298	0.353	0.587	0.574	0.744
16. *H. armigera*	Y12283	188	185	197	194	192	186	177	164	167	168	171	184	183	182	101	–	0.253	0.276	0.571	0.564	0.737
17. *C. fumiferana*	U12917	185	179	196	191	193	190	178	173	171	173	174	187	182	179	93	79	–	0.253	0.577	0.571	0.737
18. *M. sexta*	T10109	184	179	187	186	192	185	173	176	174	177	175	184	186	185	110	86	79	–	0.561	0.554	0.731
19. *C. felis*	AF053918	156	170	168	172	155	156	156	171	171	171	172	169	166	163	183	178	180	175	–	0.25	0.651
20. *C. felis*	AF053910	159	171	164	173	146	158	154	173	171	173	169	170	166	162	179	176	178	173	78	–	0.686
21. *G. morsitans*	AF252868	212	223	233	236	217	221	209	212	212	212	213	214	208	213	232	230	230	228	203	214	–

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

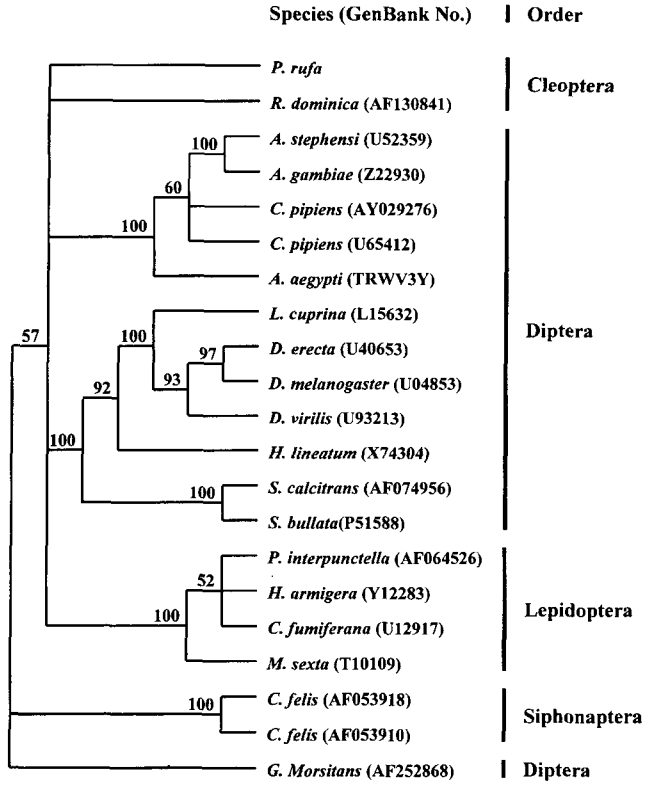

Fig. 4. A phylogenetic tree for aligned amino acid sequences of the *P. rufa* serine protease and the known serine proteases. The sequences were extracted from; *P. rufa* (this study), *R. dominica* (Zhu and Baker, 1999), *A. stephensi* (Muller *et al.*, 1993), *A. gambiae* (Muller *et al.*, 1995), *C. pipiens quinquefasciatus* (Borovsky, 1996; Borovsky *et al.*, 2001), *A. aegypti* (Kalhok *et al.*, 1992), *L. cuprina* (Casu *et al.*, 1994), *D. erecta* (Wang *et al.*, 1999), *D. melanogaster* (Wang *et al.*, 1999), *D. virilis* (Gao *et al.*, 1997), *H. lineatum* (Moire *et al.*, 1994), *S. calcitrans* (Lehane *et al.*, 1998), *S. bullata* (Borovsky *et al.*, 1996), *P. interpunctella* (Zhu *et al.*, 2000), *H. armigera* (Bown *et al.*, 1997), *C. fumiferana* (Wang *et al.*, 1995), *M. sexta* (Peterson *et al.*, 1994), *C. felis* (Gaines *et al.*, 1999), and *G. morsitans morsitans* (Yan *et al.*, 2001). The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. Outgroup was chosen as *G. morsitans morsitans* on the basis of the sequence homology by pairwise comparison.

order insects.

To confirm the expression of the *P. rufa* serine protease gene at the transcriptional level, the Northern blot analysis was carried out using the mRNA prepared from the fat body, midgut, head and light organ, respectively (Fig. 5). A hybridization signal was detected as a single band in mRNA from the whole body as a positive control and midgut but not in the fat body or light organ. As a control, 16S rRNA was used on the same blots to indicate input

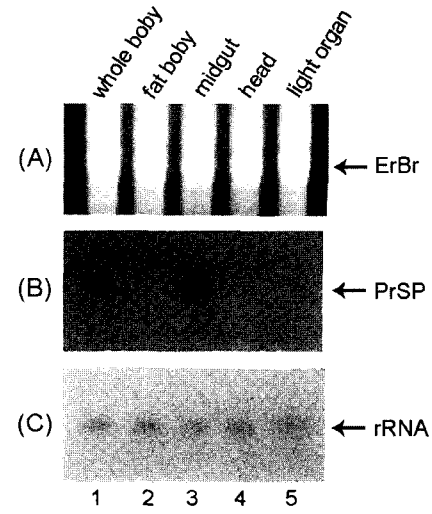

Fig. 5. Northern blot analysis of the *P. rufa* serine protease gene. Total RNAs were isolated from the whole body (lane 1), fat body (lane 2), midgut (lane 3), head (lane 4) and light organ (lane 5), respectively. The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis (A), transferred on to a nylon membrane, and hybridized with the radiolabelled *P. rufa* serine protease gene (B). The 16S rRNA gene was used as an internal loading control (C). Transcripts of the *P. rufa* serine protease gene (PrSP) are indicated on the right of panel by arrow.

RNA levels from different tissues. The Northern hybridization indicated that *P. rufa* serine protease is specifically expressed in the midgut of *P. rufa* larvae, suggesting that *P. rufa* serine protease is gut enzyme involved in digestion of dietary proteins (Yan *et al.*, 2001).

In conclusion, we report the cDNA sequence and mRNA expression of serine protease in the firefly, *P. rufa*. In this study, molecular characterization of *P. rufa* serine protease will expand our understanding of insect digestive proteases.

Acknowledgements

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References

Ashida, M. and P. T. Brey (1997) Recent advances in research on the insect prophenoloxidase cascade; in *Molecular mechanisms of immune responses in insects*. Brey, P. T. and D. Hultmark (eds.), pp. 133-172, Chapman & Hall, London.

Borovsky, D. (1996) *Culex pipiens* quinquefasciatus late trypsin precursor. *GenBank accession number U65412.*

Borovsky, D., K. Butaye and B. Daems (2001) Cloning, sequencing and characterization of *Culex pipiens quinquefasciatus* early trypsin pre-mRNA. *GenBank accession number AY029276.*

Borovsky, D., I. Janssen, J. Vanden Broeck, R. Huybrechts, P. Verhaert, H. L. De Bondt, D. Bylemans and A. De Loof (1996) Molecular sequencing and modeling of *Neobellieria bullata* trypsin. Evidence for translational control by *Neobellieria* trypsin-modulating oostatic factor. *Eur. J. Biochem.* **237**, 279-287.

Bown, D. P., H. S. Wilkinson and J. A. Gatehouse (1997) Differentially regulated inhibitor-sensitive and insensitive protease genes from the phytophagous insect pest, *Helicoverpa armigera*, are members of complex multigene families. *Insect Biochem. Mol. Biol.* **27**, 625-638.

Casu, R. E., J. M. Jarmey, C. M. Elvin and C. H. Eisemann (1994) Isolation of a trypsin-like serine protease gene family from the sheep blowfly *Lucilia cuprina*. *Insect Mol. Biol.* **3**, 159-170.

Gaines, P. J., C. M. Sampson, K. E. Rushlow and G. L. Stiegler (1999) Cloning of a family of serine protease genes from the cat flea *Ctenocephalides felis*. *Insect Mol. Biol.* **81**, 11-22.

Gao, L., S. Wang and D. A. Hickey (1997) *Drosophila virilis* Dvtry-1 trypsin precusor (Dvtry-1) and Dvtry-2 trypsin precusor (Dvtry-2) genes. *GenBank accession number U93213.*

Garnier, J., D. J. Osguthorpe and B. Robson (1978) Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* **120**, 97-120.

Gorman, M. J., O. V. Andreeva and S. M. Paskewitz (2000) Sp22D: a multidomain serine protease with a putative role in insect immunity. *Gene* **251**, 9-17.

Han, Y., C. Salazar, S. Reese-Stardy, A. Cornel, M. Gorman, F. Collins and S. Paskewitz (1997) Cloning and characterization of a serine protease from the human malaria vector, *Anopheles gambiae*. *Insect Mol. Biol.* **6**, 385-395.

Imler, J. L. and J. A. Hoffmann (2000) Signaling mechanisms in the antimicrobial host defense of *Drosophila*. *Curr. Opin. Microbiol.* **3**, 16-22.

Iwanaga, S., S. Kawabata and T. Muta (1998) New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. *J. Biochem.* **123**, 1-15.

Jiang, H. and M. R. Kanost (2000) The clip-domain family of serine proteinases in arthropods. *Insect Biochem. Mol. Biol.* **30**, 95-105.

Kalhok, S., L. M. Tabak, D. E. Prosser, A. E. R. Downe and B. N. White (1992) Trypsin-like proteinase 3A1 precursor-yellow fever mosquito. *GenBank accession number TRWV3Y.*

Kraut, J. (1977) Serine proteases: structure and mechanism of catalysis. *Annu. Rev. Biochem.* **46**, 331-358.

Kyte, J. and R. F. Doolittle (1982) A simple method for displaying hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

Lee, K. S., J. S. Bae, T. W. Goo, S. E. Kim, J. G. Kim, H. D. Sohn and B. R. Jin (2000) Analysis of expressed sequence tags of the firefly, *Pyrocoelia rufa*. *Int. J. Indust. Entomol.* **1**, 165-169.

Lee, K. S., H. J. Park, J. S. Bae, T. W. Goo, I. Kim, H. D. Sohn and B. R. Jin (2001) Molecular cloning and expression of a cDNA encoding the luciferase from the firefly, *Pyrocoelia rufa*. *J. Biotechnol.* **92**, 9-19.

Lehane, S. M., S. J. Assinder and M. J. Lehane (1998) Cloning, sequencing, temporal expression and tissue-specificity of two serine proteases from the midgut of the blood-feeding fly *Stomoxys calcitrans*. *Eur. J. Biochem.* **254**, 290-296.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacryamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Moire, N., Y. Bigot, G. Periquet and C. Boulard (1994) Sequencing and gene expression of hypodermis A, B, C in larval stages of *Hypoderma lineatum*. *Mol. Biol. Parasitol.* **66**, 233-240.

Muller, H. M., J. M. Crampton, A. della Torre, R. Sinden and A. Crisanti (1993) Temporal and spatial expression of serine protease genes in *Anopheles gambiae*. *Parasitologia Suppl.* **35**, 73-76.

Muller, H. M., F. Catteruccia, J. Vizioli, A. della Torre and A. Crisanti (1995) constitute and blood meal-induced trypsin genes in *Anopheles gambiae*. *Exp. Parasitol.* **81**, 371-385.

Paskewitz, S. M. and M. J. Gorman (1999) Mosquito immunity and malaria parasites. *Am. Entomol.* **45**, 80-94.

Peterson, A. M., C. V. Barillas-Mury and M. A. Wells (1994) Sequence of three cDNAs encoding an alkaline midgut trypsin from *Manduca sexta*. *Insect Biochem. Mol. Biol.* **24**, 463-471.

Swofford, D. L. (1990) PAUP: phylogenetic analysis using parsimony, ver. 3.0. Illinois Natural History Survey, Champaign (on disk).

Wang, S., C. Magoulas and D. A. Hickey (1999) Concerted evolution within a trypsin gene cluster in *Drosophila*. *Mol. Biol. Evol.* **16**, 1117-1124.

Wang, S., F. Young and D. A. Hickey (1995) Genomic organization and expression of a trypsin gene from the spruce budworm, Choristoneura fumiferana. *Insect Biochem. Mol. Biol.* **25**, 899-908.

Yan, J., Q. Cheng, C. B. Li and S. Aksoy (2001) Molecular characterization of two serine proteases expressed in gut tissue of the African trypanosome vector, *Glossina morsitans morsitans*. *Insect Mol. Biol.* **10**, 47-56.

Zhu, Y. C. and J. E. Baker (1999) Characterization of midgut trypsin-like enzymes and three trypsinogen cDNAs from the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Insect Biochem. Mol. Biol.* **29**, 1053-1063.

Zhu, Y. C., B. Oppert, K. J. Kramer, W. H. McGaughey and A. K. Dowdy (2000) cDNA sequence, mRNA expression and genomic DNA of trypsinogen from the Indian meal moth, *Plodia interpunctella*. *Insect Mol. Biol.* **9**, 19-26.